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Report

Intralocus Sexual Conflict Unresolved by Sex-Limited Trait Expression

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Summary

Sexually antagonistic selection generates intralocus sexual conflict, an evolutionary tug-of-war between males and females over optimal trait values [1–4]. Although the potential for this conflict is universal, the evolutionary importance of intralocus conflict is controversial because conflicts are typically thought to be resolvable through the evolution of sex-specific trait development [1–8]. However, whether sex-specific trait expression always resolves intralocus conflict has not been established. We assessed this with beetle populations subjected to bidirectional selection on an exaggerated sexually selected trait, the mandible. Mandibles are only ever developed in males for use in male-male combat, and larger mandibles increase male fitness (fighting [9, 10] and mating success, as we show here). We find that females from populations selected for larger male mandibles have lower fitness, whereas females in small-mandible populations have highest fitness, even though females never develop exaggerated mandibles. This is because mandible development changes genetically correlated characters, resulting in a negative intersexual fitness correlation across these populations, which is the unmistakable signature of intralocus sexual conflict [1]. Our results show that sex-limited trait development need not resolve intralocus sexual conflict, because traits are rarely, if ever, genetically independent of other characters [11]. Hence, intralocus conflict resolution is not as easy as currently thought.

Results and Discussion

Sexually antagonistic selection on shared characters generates intralocus sexual conflict [1–3], and because shared traits are expressed in both sexes, selection in one sex can impede the evolution of the character toward the fitness optima of the other sex. However, sex-limited trait development (through any mechanism) is frequently claimed to extinguish intralocus sexual conflict—but, although widely asserted [1–8], this has never been experimentally tested. We tested this fundamental claim by experimentally evolving the size of an exaggerated sexually selected character in replicate beetle populations. Exaggerated sexually selected traits include ornaments and weapons that are used to attract or compete for mates [12]. These enlarged characters generally provide fitness advantages to males, but not females, and as a result are frequently only highly developed in males (e.g., peacocks' tails) [12]. Enlarged sexual traits offer an ideal means of assessing the effect of sex-limited trait expression on intralocus sexual conflict because of the clear sexual differences in optimal trait size.

Male broad-horned flour beetles (*Gnatocerus cornutus*) develop massively enlarged mandibles that are used in male-male combat, and males with larger mandibles are better fighters [9, 10], but females lack this character exaggeration completely (Figure 1) [9, 10, 13]. All close relatives of this species are sexually monomorphic for mandible size [13], suggesting that dimorphic mandibles are derived and larger male mandibles have evolved through positive selection for increased mandible size in males (i.e., there has been sexually antagonistic selection on mandible size). This is potentially important because there is some evidence that characters can be sex limited from their origin and hence may not be subject to antagonistic selection [14].

We established replicate beetle populations and bidirectionally selected on male mandible size, while maintaining matching control lines, to generate six experimental populations: two selected for enlarged male mandibles (L), two for reduced male mandibles (S), and two controls with no selection on male mandible size (C) [9]. Previous work has shown that selection rapidly altered male (but not female) mandible size, and males with enlarged mandibles were more successful fighters [9, 10]. Here we assessed the impact that selecting on male mandible size has had on female fitness. If the evolution of sex-limited mandible exaggeration has quelled intralocus sexual conflict over optimal mandible size, which it is claimed to do [1–8], artificially selecting on mandible size in males should not alter female fitness. However, if we find that enlarging male mandibles reduces female fitness, then sex limitation has not resolved intralocus conflict over mandible size. This is because previous work has shown that larger (smaller) mandibles increase (decrease) a major male fitness component in these experimental populations [9, 10], and, as we confirm below, the primary determinant of male fitness (male mating success) and a negative intersexual correlation for fitness is the unequivocal indicator of intralocus sexual conflict [1].

After 12 generations of selecting on male mandible size, we first had to establish that mandible size differed as previously reported, and we found that it did (see Experimental Procedures). We then conducted a mating assay using the selection lines to see whether mandible size influenced male mating success against tester males and found that males with larger mandibles (L populations) were more likely to copulate than males with small mandibles (S populations). Control males were intermediate (male mating success [percent securing copulation ± standard error, SE]: L = 75.0 ± 5; C = 47.5 ± 2; S = 17.5 ± 2; $F_{2,3} = 66.2$; p = 0.003; Fisher's PLSD: L > C > S [all $p < 0.012$]). Thus, larger mandibles increase male fighting [9, 10] and mating success, and, consistent with inferences based on the derived nature of the exaggerated male

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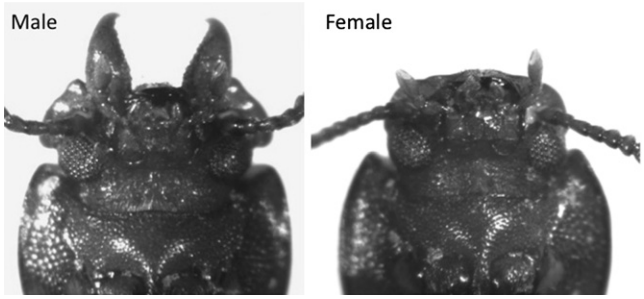

Figure 1. The Substantial Sexual Dimorphism Seen in Beetle Mandible Size

Left image shows the male head with enlarged mandibles; right image shows a female's head where the mandible exaggeration is completely lacking. Mandibles are used in male-male competition, and males with larger mandibles have higher fitness.

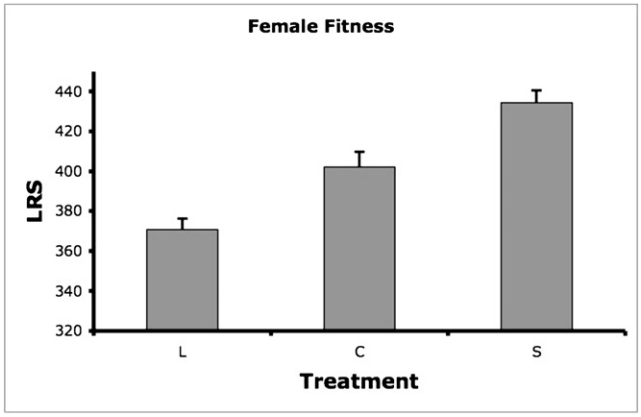

Figure 2. Female Fitness

Female fitness measured as lifetime fecundity (LRS: mean ± SE) in the experimental populations (L = populations with artificial selection for larger male mandible size; C = control populations where there was no artificial selection imposed on male mandible size; S = populations with artificial selection for smaller male mandible size). There was a significant effect of selection (treatment) on LRS (general linear model [GLM] of treatment [L,C,S] effects using population means: $F_{2,3} = 22.2$; $p = 0.014$; post hoc tests [Fisher's PLSD] revealed that L < C < S [all $p < 0.04$]).

mandibles (selection has favored larger male mandibles; see above), males with larger mandibles have higher fitness.

Females from each experimental (and control) population were then experimentally paired with males from control populations. The use of only control males standardizes for male effects on female fitness. We then assessed female lifetime fecundity (lifetime reproductive success, LRS), a standard measure of female fitness [15–17], and also recorded longevity following standard protocols [18]. We found that female LRS was lower in the populations selected for larger male mandibles and highest in populations selected for smaller male mandibles (controls were intermediate; Figure 2). Thus, selecting on traits only exaggerated in males (and not exaggerated in females at all) has impacted on female fitness, and because these beetles are stored product pests [13], this is unlikely to be a simple laboratory artifact, because we were replicating their "normal" habitat in the lab.

We then assessed several potential mechanistic explanations for the differences in LRS across our experimental treatments. It was possible that female LRS was influenced by female survival, mass, abdomen size (the body region where eggs are produced and stored), or the size of the eggs females produced, and there is evidence that each of these parameters influences female fitness [19–21]. We tested for differences in each of these parameters across our populations and found that only the size of the female abdomen differed significantly, with the differences consistent with the LRS relationship (Figure 3): females from the populations selected for larger male mandibles had evolved relatively small abdomens, whereas females from the small-mandible populations had larger abdomens (Figure 3D). Because abdomen size determines the number of eggs a female insect can carry [19, 20], this provides a proximate explanation for the LRS reduction. Female longevity, total body mass, and egg volume had not evolved and did not differ between treatments (Figure 3). We note that females had not evolved exaggerated mandibles, because there is a genetic decoupling of mandibles across the sexes (Figure 1) [9, 10, 13]. Nevertheless, selection on male mandible size clearly affected female fitness. Because larger (smaller) mandibles increase (decrease) male fitness but, as we find here, decrease (increase) female fitness, we have documented the negative intersexual correlation that is the explicit signature of intralocus sexual conflict [1]. Our results therefore unequivocally demonstrate that sex-limited mandible exaggeration has not resolved intralocus sexual conflict over mandible size. Selection for weapon enlargement has generated correlated changes in beetle body form as a result of the need to bear the enormously developed mandibles—males evolving larger mandibles also have smaller abdomens, for example [9]—and this impacts on female fitness, even though females do not develop the exaggerated mandibles; in addition, reassuringly for our interpretation, selection for reduced weapons resulted in increased female fecundity.

In sum, our results indicate that whether or not sex-limited trait development extinguishes intralocus sexual conflict depends upon genetic architecture. Genetic covariance in male characters, coupled with intersexual genetic correlations for traits that are not sex limited in expression, can merely cause the focus of intralocus sexual conflict to shift. In the beetle populations, this focus has moved from the mandibles themselves to secondary characters that are associated with mandible enlargement. However, mandible exaggeration is still the root cause of the negative intersexual fitness associations we found, even though this exaggeration is sex limited. The view that sex-limited trait development resolves intralocus sexual conflict is predicated on the assumption that traits are genetically independent of each other, which is frequently not true [11, 22], and serves to highlight the problem of deciding what a "trait" really is [11]. In any case, like mutation accumulation in the unselected sex [23], genetic architecture can provide a general barrier to intralocus conflict resolution.

Clear evidence of ongoing intralocus sexual conflict has now been documented in many groups, including insects, reptiles, birds, and mammals [24–28]. This suggests that intralocus conflict continually arises despite the near-ubiquity of sexual dimorphism [5, 29]. Our study finds that sexual selection for an exaggerated male trait generates intralocus sexual conflict through changes in genetically correlated characters that are not sex limited in their development. This demonstrates that, even with sex-limited trait expression, intralocus conflict resolution may not be as easy as currently thought (see also [4, 23, 30]). Additionally, it has been suggested that intralocus sexual conflict may not be evolutionarily important because conflicts

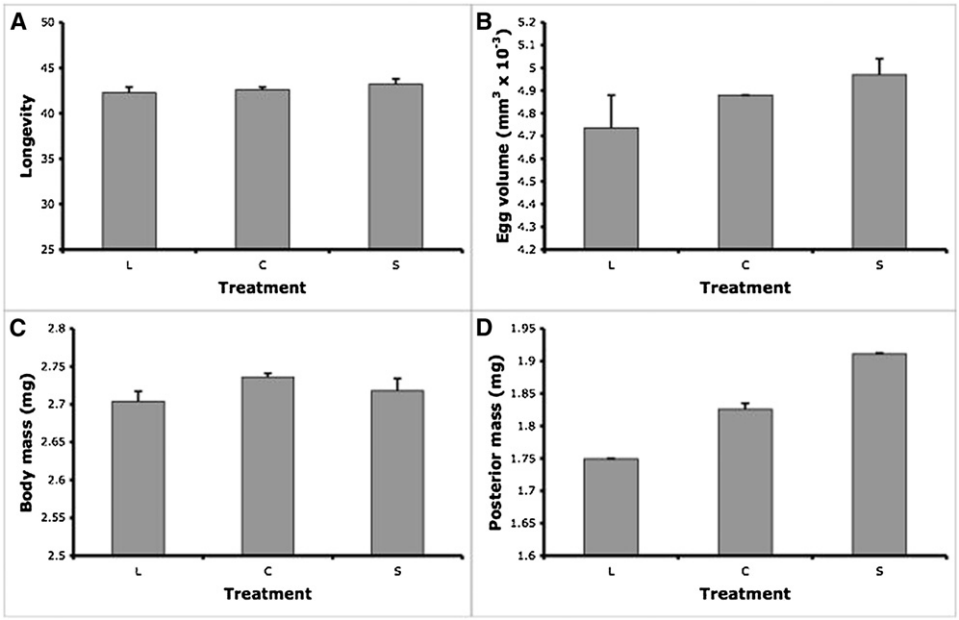

Figure 3. Female Trait Evolution

Characters that potentially provide a proximate explanation for the differences in female LRS across the experimental populations (L = populations with artificial selection for larger male mandible size; C = control populations where there was no artificial selection imposed on male mandible size; S = populations with artificial selection for smaller male mandible size). Separate GLMs were used to assess each variable with selection (treatment: L,C,S) as a fixed effect.

(A) Female longevity (weeks).

(B) Egg volume ($mm^3 \times 10^{-3}$).

(C) Female body mass (mg).

(D) Posterior mass (mesothorax, metathorax, and abdomen; mg). Only the size of the female rear portion (posterior mass, D) significantly differed across treatments, and this difference remains statistically significant with sequential Bonferroni correction ($F_{2,3} = 213.6$; $p < 0.001$; post hoc tests [Fisher's PLSD] show that L < C < S [all $p < 0.002$]). None of the other characters measured significantly differed across treatments (all $F < 1.8$; all $p > 0.30$). Note that the posterior mass differences precisely mirror the fitness differences (Figure 2). Data are means ± SE.

can be defused by sex-limited trait expression, allowing the sexes to reach their respective fitness optima [6, 7]. By providing direct evidence that sex limitation does not always resolve intralocus sexual conflict over the sex-limited trait, our study indicates that this conflict may be more important than currently appreciated and, as with so many evolutionary conflicts, apparent resolution at one level can disguise additional discord that arises elsewhere.

Experimental Procedures

Animals and Culturing

The *G. cornutus* beetle culture originated from adults collected in Miyazaki City (31°54', 131°25'), Japan, and has been maintained in the laboratory of the National Food Research Institute, Japan, for about 50 years on whole meal enriched with yeast as food. We performed all rearing and experiments in a chamber maintained at 25°C, 60% relative humidity, with a photoperiod cycle of 16:8 hr light:dark conditions.

Artificial Selection

The artificial selection protocol was described in detail by Okada and Miyatake [9]. Briefly, we established lines selected for long (two replicates) and short (two replicates) male mandibles with unselected control lines (two replicates). At each generation, 12 males with the shortest and longest mandibles were selected from a random collection of 100 males in each line of long- and short-mandible selection regimes, respectively, and 12 males were randomly selected in each control line. They were mated with females chosen randomly from their own line to propagate each line. In both replicates, mandible length responded to selection in both directions and clearly diverged between selection regimes after 10 generations of selection [9]. The following experiments were performed on generation 12 individuals, but we first had to establish that mandible size differed as previously reported, and we found that it did. Using a general linear model (GLM) with treatment (selection regime: L = selection for larger mandibles, C = control [no artificial selection on mandibles], S = selection for smaller mandibles) as a fixed effect, there was a significant effect of selection on mandible size (using population as the replicate: mean mandible length [mm ± SE] L = 0.45 ± 0.002; C = 0.37 ± 0.008; S = 0.26 ± 0.002; $F_{2,3} = 359.8$; $p < 0.001$; Fisher's PLSD post hoc tests showed L > C > S [all $p < 0.002$]). This is consistent with previous assessment two generations earlier [9].

Body Size and Shape

We measured mandible length in males and mass of overall body and posterior of the body (i.e., mesothorax, metathorax, and abdomen) in females. Twenty-five males were randomly chosen from each line and frozen at −20°C immediately after emergence. Mandible length was measured following Okada and Miyatake [9], and the whole and posterior of their bodies were weighed to the nearest 0.01 mg on an electronic balance (Mettler-Toledo AG, Laboratory and Weighing Technologies). The whole and posterior of the body were also weighed at death in females used for the following experiment.

Male Mating Success

Males from the six experimental populations (L, C, S) were used to assess male mating success as a function of mandible exaggeration. We staged 20 contests per population (n = 120 in total) in which experimental males competed against a male from the stock culture in the following manner. We placed a plastic cup (5 mm diameter, 5 mm height) with an excess of the culture medium as the territory into a plastic container (35 mm diameter, 15 mm height). We then placed one female from the stock culture into the container, and, 2 hr after this introduction, we simultaneously introduced two males (one experimental population male and one stock male) into the container. Triads were then continuously observed until copulation ended. We recorded which male copulated (males were marked with white

or pink spots [Mitsubishi Paint-Marker] on their elytra; in half of the trials, focal males were white, and in half of the trials, focal males were pink) and could then compare the proportion of copulations obtained by males from each experimental treatment (GLM with population as the replicated factor and treatment as a fixed factor). Experiments were conducted 15–20 days after emergence, and males had been housed alone so they could not interact with conspecifics.

Female Fitness

We assessed the lifetime fecundity, egg size, and longevity of 45 females collected from each line. Following Tsuda and Yoshida [18], we placed a virgin female and a virgin male, both of which were less than a week post-emergence, in a glass vial (40 mm high, 15 mm diameter) containing food (4 g). All females were paired with males from the corresponding control line (assigned a priori following the propagation regime: three populations [one from each treatment] propagated each day) to control for male effects. If a male died, it was replaced with another male of the same age. Food was replaced once a month, and female survival was assessed weekly. To measure egg size, we randomly chose five eggs laid by each female in the first week after pairing. The length (L) and width (W) of the egg were measured to the nearest 0.01 mm, and the volume (V) of the egg was calculated using the formula $V = (\pi LW^2)/6$ and then averaged across five eggs within each female. All analyses were conducted on population (line) means using GLM with female selection regime as a fixed effect and using SPSS for Windows version 14. However, even if we more conservatively use paired t tests to assess differences in line mean female LRS (because selection lines were matched to particular control lines), our conclusions remain unchanged (paired comparison of female LRS: L < S – df = 1; paired t = −115.36; p = 0.006).

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